

Genetic diversity of ST5 *Staphylococcus aureus* isolated from swine veterinarians in the USA

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Abstract

The term livestock associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) has been synonymous with sequence type ST398 since the identification of this lineage of MRSA in Holland. Subsequent research indicates broader genetic diversity of MRSA strains in swine, with MRSA variants belonging to MLST sequence type ST9, and ST5 also being reported in studies of swine in various countries. Unlike ST398, considered a novel sequence type, the ST5 lineage has long been associated with human MRSA infections, and particularly the USA100 group (defined by *smaI* PFGE).

We investigated the prevalence of *S. aureus*, and associated health events, in swine veterinarians in US and evaluate the genetic diversity of isolates using *spa* typing, MLST and PFGE. The ST398 and ST5 were the most prevalent *spa* types and 60 (18%) of 325 MSSA isolates and 6 (19%) of 32 MRSA isolates detected were *spa* type t002 (ST5). *SmaI* PFGE analysis of 23 isolates indicated that t002 isolates from the swine veterinarians were not clonal. Given previous reports of t002-ST5 MRSA in North American pigs, our findings of diverse ST5 *spa* types as well as genetic diversity within *spa* type t002, suggest that *S. aureus* variants of the ST5 lineage may be livestock associated. These organisms need to be more broadly characterized to understand their potential implications for human health.

Introduction

Livestock associated *Staphylococcus aureus* (LA-MRSA) has been deemed an emerging zoonotic pathogen in food animals, especially in pigs (Voss et al. 2005). Among early studies, the first and most predominant LA-MRSA type was sequence type (ST) 398 in Europe and North America while ST 9 variants appear to be predominant in Asia (Lewis et al. 2008; Smith et al. 2009; Cui et al. 2009; Asai et al. 2012). Various genetic studies are revealed more genetic diversity in LA-MRSA using molecular epidemiologic characteristics. There is considerable evidence for pig-to-human transmission of ST398 in occupationally exposed people (Garcia-Graells et al. 2012).

Recently, ST5 MRSA, well known as most globally disseminated hospital associated MRSA lineages, has been identified from pigs, retail meat and farm workers in North America (Khanna et al. 2008; Frana et al. 2013). The occurrence of ST5 variants in swine is arguably more concerning than ST398 MRSA, which to date have had minimal public health impact despite high prevalence of exposure in people with livestock contact.

The aim to this study was to compare ST5 MRSA and MSSA strains from US swine veterinarians using pulse-field gel electrophoresis (PFGE). The isolates were obtained as part of a longitudinal study of *S. aureus* nasal colonization in swine veterinarians. We postulated that a high degree of clonality among these isolates would suggest recent emergence of this lineage in the livestock sector, whereas greater diversity would suggest longer term association of ST5 with swine, or exposures from other sources.

Materials and methods

In an ongoing longitudinal study, nasal swabs are being collected from 67 US swine veterinarians. Participants mail self-collected nasal swabs monthly to the laboratory, along with information on recent animal exposure and health events. The most prevalent MLST types of *S. aureus* isolated in the first 7 months of the study (July 2012 – January 2013) were ST398, ST5 and ST9, with ST398 and ST5 the most common among MRSA isolates. The veterinarians reside in 15 major swine producing states of the USA and typically interact with diverse swine populations in their work.

Pulsed-field gel electrophoresis (PFGE)

To evaluate the diversity of ST 5, the 23 ST5 *S. aureus* isolates (3 MRSA and 20 MSSA isolates) determined to be *spa* type t002 (the predominant *spa* type among ST5 isolates) PFGE was performed using the *smaI* restriction enzyme (McDougal

et al. 2003). Visualized images were analyzed using BioNumerics software (Version 5.0) and PFGE types were defined using a similarity coefficient of 85% and USA 100 to USA 800 strains were included as references.

Results

Overall in first 7 months of the longitudinal study, 60 (18.5%) of 325 MSSA isolates and 6 (18.8%) of 32 MRSA isolates detected were spa type t002 (ST5). Other ST5 MSSA spa types found included t045 (14), t062 (6), t570 (3) and t2049 (1). We purposively selected 23 of the t002_ST5 isolates to attempt to maximize diversity of isolates among months and participants. For participants from whom t002-ST5 was isolated on more than one occasion, we sampled the first and last isolates available.

PFGE analysis showed that the 23 t002-ST5 isolates from swine veterinarians were not uniform, and therefore unlikely to share a recent clonal relationship. Fifteen distinct pulsotypes were seen, and eight isolates were classified as USA100, a common pulsotype in human clinical infections. Among participants with two MSSA isolates analyzed, PFGE patterns from one were exactly identical, while isolates from another individual participant showed only 85% similarity. For one participant with multiple MRSA isolates, the PFGE patterns were identical although there was a 6-month interval between the sampling events.

Discussion

Recently, ST5 has been reported backyard-raised pigs in Michigan (Gordoncillo et al. 2012) and from retail pork or pork farms in U.S (Molla et al. 2012) and even pigs and pig farmers in Canada (Khanna et al. 2008). In a pilot study of *S. aureus* colonization of pigs on 2 farms in Minnesota (unpublished), we also found the predominant spa types to be t034 (ST398), and t002 (ST5), comprising 37% and 29% of isolates respectively (all MSSA). Originally ST 5 is a common hospital associated MRSA type, mostly related to nosocomial infections. Although the isolates we analyzed were from human nasal swabs, our ongoing studies (unpublished) and a recent study from Holland (Verkade et al. 2013) suggest that most *S. aureus* isolates from swine veterinarians originate from occupational exposures. The diversity of PFGE types among these isolates, together with increasing reports of ST5 *S. aureus* from pigs in diverse locations suggest that this lineage may have a long association with swine rather than reflect a recent event of interspecies transmission. Further evaluation of human and swine ST5 *S. aureus*, particularly with respect to the presence of virulence determinants, is required to evaluate the potential occupational health risk associated with these organisms.

Conclusion

The presence of diversity in ST5 *S. aureus* isolates from swine veterinarians on PFGE suggests that ST5 MSSA and MRSA **have colonized this population** having a long association with pigs.

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